PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 24 January 2001 (24.01.01)	
International application No. PCT/US00/08282	**Applicant's or agent's file reference** 1530031PC02
International filing date (day/month/year) 24 March 2000 (24.03.00)	**Priority date** (day/month/year) 26 March 1999 (26.03.99)
Applicant WHEELER, Carl, J.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

25 October 2000 (25.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Philippe Bécamel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number
WO 00/57917 A3

(51) International Patent Classification[7]: A61K 47/18, 39/39, A61P 31/04, 31/12 // 33/00, 35/00, C07C 217/28

(21) International Application Number: PCT/US00/08282

(22) International Filing Date: 24 March 2000 (24.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/126,340 26 March 1999 (26.03.1999) US

(71) Applicant *(for all designated States except US)*: **VICAL INCORPORATED** [US/US]; Suite 100, 9373 Towne Centre Drive, San Diego, CA 92121 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **WHEELER, Carl, J.** [US/US]; 14101 Arbolitos Drive, Poway, CA 92064 (US).

(74) Agent: **NAN, Wu et al.**; Pillsbury Madison & Sutro LLP, 50 Fremont Street, San Francisco, CA 94105 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

(88) Date of publication of the international search report:
4 January 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/57917 A3

(54) Title: ADJUVANT COMPOSITIONS AND METHODS FOR ENHANCING IMMUNE RESPONSES TO POLYNUCLEOTIDE-BASED VACCINES

(57) Abstract: The invention provides adjuvants, immunogenic compositions, and methods useful for polynucleotide-based vaccination and immune response. In particular, the invention provides an adjuvant of cytofectin:co-lipid mixture wherein cytofectin is GAP-DMORIE.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT 00/08282

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/18 A61K39/39 A61P31/04 A61P31/12 //A61P33/00, A61P35/00,C07C217/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, BIOSIS, MEDLINE, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 19675 A (VICAL INC) 5 June 1997 (1997-06-05) the whole document ---	1-66
A	NORMAN J A ET AL: "Development of improved vectors for DNA-based immunization and other gene therapy applications" VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 8, 1 June 1997 (1997-06-01), pages 801-803, XP004075655 ISSN: 0264-410X the whole document --- -/--	1-66

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 2000	30/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Niemann, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WHEELER C J ET AL: "CONVERTING AN ALCOHOL TO AN AMINE IN A CATIONIC LIPID DRAMATICALLY ALTERS TO CO-LIPID REQUIREMENT, CELLULAR TRANSFECTION ACTIVITY AND THE ULTRASTRUCTURE OF DNA-CYTOFECTIN COMPLEXES" BIOCHIMICA ET BIOPHYSICA ACTA,NL,AMSTERDAM, vol. 1280, no. 1, 1996, pages 1-11, XP002035803 ISSN: 0006-3002 the whole document	1-66
A	WHEELER CARL J ET AL: "A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 93, no. 21, 1996, pages 11454-11459, XP002150135 1996 ISSN: 0027-8424 cited in the application the whole document	1-66

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern...al Application No
PC... 00/08282

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9719675 A	05-06-1997	CA 2237316 A	05-06-1997
		EP 0863749 A	16-09-1998

09/19576014

PATENT COOPERATION TREATY

REC'D 11 JUL 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1530.03 PCO2		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/08282	24/03/2000	26/03/1999
International Patent Classification (IPC) or national classification and IPC A61K47/18		
Applicant VICAL INCORPORATED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 12 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/10/2000	09.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hornich, E Telephone No. +49 89 2399 8721

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/08282

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-6,8-24,26-38,40-45, 47,48,50	as originally filed	
7,25,39,46,49	with telefax of	09/03/2001

Claims, No.:

1-66	with telefax of	09/03/2001

Drawings, sheets:

1/14-14/14	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 27 - 59 (with regard to industrial applicability only); 51.

because:

   ☒ the said international application, or the said claims Nos. 27 - 59 (with regard to industrial applicability, see sep. sheet 2.) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 51 (see sep. sheet 3.) are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;

citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-50, 52-66 (see separate sheet 5.)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-50, 52-66 (see separate sheet 6.)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26, 60-66 (see separate sheet 7.2); 27-59 (see separate sheet 2. and 7.1)
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION I

1. The amended claim 52 contains subject-matter which extends beyond the content of the application as originally filed.
   Originally, the subject-matter of claim 52 related to the '*administration mediated by a Biojector® 2000*', which was to amend, as trademarks are not desirable (the PCT-Guidelines, C-III, 4.5b).
   Accordingly, the Applicant introduced '*a CO_2 powered jet injection system*' in order to replace *'Biojector® 2000'*, which is, however, a generalisation, not supported by the description, and therefore an *extent of the subject-matter of the application as originally filed.*
   Therefore, the amendment concerning claim 52 **does not meet the requirements** of **Art. 19(2)** and **34(2)b PCT**.

SECTION III

2. Claims 27 - 59 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

3. Claim 51 lacks clarity (**Art. 6 PCT**). From the wording "The method of any one of claim 28" it is not evident which preceding claim(s) claim 51 refers to.

SECTION V

4. Reference is made to the following documents:

   D1: WO 97 19675 A
   D2: NORMAN J A ET AL: 'Development of improved vectors for DNA-based immunization and other gene therapy applications' VACCINE,GB,BUTTERWORTH SCIENTIFIC. GUILDFORD, vol. 15, no. 8, 1 June 1997 (1997-06-01), pages 801-803, ISSN: 0264-410X

D3: WHEELER C J ET AL: 'CONVERTING AN ALCOHOL TO AN AMINE IN A CATIONIC LIPID DRAMATICALLY ALTERS TO CO-LIPID REQUIREMENT, CELLULAR TRANSFECTION ACTIVITY AND THE ULTRASTRUCTURE OF DNA-CYTOFECTIN COMPLEXES' BIOCHIMICA ET BIOPHYSICA ACTA,NL,AMSTERDAM, vol. 1280, no. 1, 1996, pages 1-11, ISSN: 0006-3002

D4: WHEELER CARL J ET AL: 'A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung.' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 93, no. 21, 1996, pages 11454-11459, 1996 ISSN: 0027-8424 cited in the application

4.1 The invention within **D1** provides cytofectin (=cationic lipids that enhance cell transfection, p. 1, l. 15-30) compositions comprising co-lipids (e.g. phospholipids: DOPE: dioleoylphosphatidylethanolamine) and polynucleotides or peptides aimed at facilitating the transport of the macromolecules through the plasma membrane of cells and into the cytoplasm. GAP-DMORIE is comprised within the Markush-Formula (see claim 10), but not explicitly mentioned (p. 2, l. 25/26; p. 13, l. 3-5 and l. 15; p. 14, l. 13-19; p. 23, l. 4-17;. p. 27, l. 20-29; Ex. 8).

4.2 **D2** discloses the enhancing effect of the cationic lipid GAP-DLRIE (cytofectin which is related to GAP-DMORIE, see 2/14 of the 'Drawings' of present application) respectively GAP-DLRIE / DOPE (cytofectin - co-lipid - complex) on the uptake and expression of plasmid DNA (see abstract; p. 802, right col., "Plasmid DNA vector delivery": l. 1-3 and l. 23-26; p. 803, left col., l. 13-16).

4.3 Document **D3** discloses that the cytofectin GAP-DMRIE (=[SPEC0803]AE-DMRIE, N-(2- aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide) is superior to DMRIE (hydroxyethyl-analogue) in mediating in vitro cellular transfection, with and without addition of the co-lipid DOPE (p. 6, left col., 2nd paragraph; p. 10, left col., 2nd and 3rd paragraph).

4.4 **D4** investigates the effect of various cytofectins (in combination with the co-lipid DOPE) on the delivery and expression of plasmid DNA. GAP-DLRIE emerged as one of the most potent compounds (see p. 11456, left col., 'Results'; p. 11457, right col., 'Discussion', 1st paragraph).

5. Novelty (**Art. 33(2) PCT**)

According to above-cited documents **D1** to **D4**, compositions comprising GAP-DMORIE [N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradecenyloxy)-1-propan-aminium bromide] and (a) co-lipid(s) have not yet been described.

Therefore, the **subject-matter of claims 1 - 50 and 52 - 66 is novel** with regard to **Art. 33 (2) PCT**.

6. Inventive Step (**Art. 33(3) PCT**)

Within the present application, comparative data is provided showing the unforeseeable superior effect of GAP-DMORIE on antibody stimulation, after application of formulations comprising GAP-DMORIE, a co-lipid and plasmid DNA, when compared to related cytofectins (see Fig. 3, 3/14; Fig. 4, 4/14). The increase in the amount of antibodies is highly desirable, since insufficient or suboptimal humoral response is a major problem frequently encountered in the course of polynucleotide-based vaccination; thus, quantity of immunogen and the frequency of administration can be reduced, the benefits of which are evident (see p. 2, l. 5-17 and p. 3, l. 8-17 of present application).

Therefore, an **inventive step** can be **acknowledged** (**Art. 33(3) PCT**) for claims 1 - 50 and 52 - 66.

7. Industrial Applicability (**Art. 33(4) PCT**)

7.1 For the assessment of the present claims 27 - 59 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

7.2 The requirements concerning the industrial applicability are as well fulfilled for the claims 1 - 26 and 60 - 66.

SECTION VIII

8. The Chemical Abstract Index Name of '**GAP-DMORIE**' is '*1-Propanaminium, N-(2-aminoethyl)-N,N-dimethyl-2,3-bis[(9Z)-9-tetradecenyloxy]-,**bromide***', registered under the *CAS-RN 299207-54-8*.

Therefore, all passages within the description and the claims referring to '*salts* of GAP-DMORIE' involve unclarity in the sense of **Art. 6 PCT**, as 'the salt' of a compound already being a salt is scarcely conceivable.

In the light of the above-mentioned CAS-definition of GAP-DMORIE, the originally filed claim 1 as well as all further claims involving 'GAP-DMORIE' appear to be correct.

9. The chemical name of DPyPE, '*1,2-diphytam**o**yl-sn-glycero-3-phosphoethanolamine*', given within the claims does not correspond to the nomenclature given within the description (see p. 4, l. 9, '*1,2-diphytan**o**yl-sn-glycero-3-phosphoethanolamine*').

were determined from serum samples at various time points. The lines represent average anti-NP titers + S.E.M. (n = 4 - 10 mice per time point).

Figures 8A, 8B, and 8C illustrate that pDNA formulated with Vaxfectin induces cytotoxic T lymphocyte (CTL) responses that are as robust as those induced with naked pDNA. (A) Mice received bilateral intramuscular injections of 5 μg VR4700 plasmid DNA encoding influenza nuclear protein (NP) in 50 μl PBS per muscle on day 0, 21, 42 and 63. A second set of mice received the same pDNA dose formulated with Vaxfectin at the indicated pDNA:cationic lipid molar ratios. (B) Mice received bilateral intramuscular injections of 1 or 25 μg VR4700 plasmid in 50 μl PBS per muscle on day 0, 21, 42 and 63. A second set of mice received the same pDNA doses formulated with Vaxfectin at a pDNA:cationic lipid molar ratio of 4:1. (C) Mice received bilateral intramuscular injections of 5 μg VR4700 plasmid in 50 μl 150 μM Nap per muscle on day 0 and 2 1. A second set of mice received the same pDNA dose formulated with Vaxfectin at a pDNA:cationic lipid molar ratio of 4:1. All CTL assays were performed 4-4.5 months after the first injection. The lines represent average specific lysis (n = 4 - 5 mice per group).

Figure 9 illustrates the effect of Vaxfectin on β-galactosidase (β-Gal) expression in muscle. Mice received intramuscular injections of 5 μg naked VR1412 plasmid encoding β-galactosidase. A second group of mice was injected with 5 μg VR1412 formulated with Vaxfectin at a pDNA:cationic lipid molar ratio of 4:1. At the indicated time points, quadriceps muscles were harvested and assayed for β-Gal activity. The lines represent average reporter gene expression per muscle ± S.E.M. (n = 10 - 20 muscles per group).

Figure 10 illustrates that Vaxfectin enhances Immoral immune response in rabbits. Total IgG antibody titers in rabbit serum after i.m. injection of VR4700 plasmid DNA encoding influenza nuclear protein (NP) are shown. New Zealand White rabbits (5-6 months old) received a single unilateral injection of either 150 μg VR4700 plasmid alone or formulated with (pDNA:cationic lipid = 4:1 molar ratio) in 300 μl PBS. In one group of animals (triangles), both pDNA and pDNA-Vaxfectin were injected using needle and syringe. In another group of rabbits

DMORIE + 60 μg DPyPE per mouse) did not appear to produce discomfort or result in any adverse reactions when injected into mouse muscle.

Preparation of pDNAs

The VR4700 plasmid was prepared using standard techniques known in the art. Briefly, VR1255, an optimized plasmid encoding firefly luciferase (Hartikka, J., *et al., Human Gene Therapy 7*:1205-1217 (1996)), had the coding sequence for influenza nuclear protein (NP) inserted in place of the luciferase coding sequence. The influenza nuclear protein sequence was derived from a plasmid termed nCMVint-tpaPRNP (Vahlsing, L., *et al., J. Immunol. Methods 174*:11-22 (1994)). More specifically, the VR4700 plasmid was created via the following procedure. The VR1255 plasmid was digested with *Acinetobacter calcoacetius* restriction enzyme (Acc I) + *Bacillus amyloliquefaciens* HI restriction enzyme (Bam HI), then the ends were blunted with Klenow, thus affording the desired vector fragment. The nuclear protein coding sequence was obtained by digesting nCMVintTPAPRNP with Acc I + *Escherichia coli* I restriction enzyme (Eco RI), and blunting the ends with Klenow. Both the vector fragment and the insert fragment were purified, then ligated with T4 DNA ligase. The ligation products were transformed in *E. coli* to kanamycin resistance, after which suitable plasmid bearing clones were identified based on restriction digest profiles. Standard cell culture techniques were used to expand a suitable clone, from which the plasmid was initially isolated and purified using well known, commercially available technology (Qiagen, Valencia, CA).

VR1412 LacZ plasmid was constructed by subcloning a cytoplasmic-targeted β-galactosidase gene into the VR1012 vector (Doh, S.G., *et al., Gene Therapy 4(7)*:268-263 (1997)). The VR1012 backbone vector contains the human cytomegalovirus (CMV) immediate early 1 promoter/enhancer, CMV intron A, bovine growth hormone terminator and kanamycin resistance gene (Hartikka, J., *et al.*, *Human Gene Therapy 7(10)*:1205-17 (1996)).

VR5900 is a pDNA encoding hen egg lysozyme. For construction of this pDNA, gallus lysozyme cDNA was synthesized with overlapping oligonucleotides using Deep Vent DNA polymerase (NEB, Boston, MA). The nucleotide sequence

[c] Mice received bilateral intramuscular injections of 5 μg VR4700 pDNA in 50 μl PBS, either alone or formulated with Vaxfectin at 4:1 pDNA:cationic lipid molar ratio. Identical boost injections were given at three weeks and at nine months. Mice were sacrificed eleven months after the start of the experiment (two months after the second boost injection). Antibody titers were measured from terminal bleeds and anti-NP secreting cells were quantified from bone marrow.

[d] Significantly different from pDNA control value ($p = 0.029$, Mann-Whitney rank sum test).

The data from the ELISPOT assays indicate that the use of Vaxfectin increases the number of antigen specific plasma cells in the bone marrow. This increase in plasma cells may be due to the adjuvant properties of the pDNA-lipid complexes. Injection of blank pDNA complexed with a cationic lipid into the peritoneum of murine ovarian tumor bearing C3H/HeN mice induces the production of interleukin-6 (IL-6), interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (Horton, H.M., *et al, J. Immunol. 163(12)*:6378-6385 (1999)). These cytokines were not induced in mice treated with pDNA or lipid only, suggesting that the pDNA-lipid complexes are immunostimulatory *in vivo*. The inummostimulatory properties of pDNA-lipid complexes were also reported for experiments in which mice were injected intravenously with pDNA complexed with cationic lipid (Dow, S.W., *et al, J. Immunol. 163(3)*:1552-1561 (1999)). As for intraperitoneal and intravenous injection of pDNA-lipid complexes, intramuscular injection of pDNA-Vaxfectin may also induce cytokines, including IL-6, a cytokine that promotes the differentiation of activated B cells to plasma cells. Thus, the pDNA-Vaxfectin complexes may indirectly enhance antibody titers by increasing the number of antibody producing B cells.

It is also possible that components of Vaxfectin might mimic naturally occurring mitogens that can directly stimulate polyclonal expansion of B cells. This could enhance the specific immune response against the transgene expressed by the muscle cells by increasing the number of responding B cells. Thus, increased transfection of APCs or delivery of pDNA to the draining lymph nodes with transfection of cells in the lymph nodes, muscle damage resulting in increased availability of soluble antigen and the immunostimulatory properties of the pDNA-Vaxfectin complexes could each contribute to the adjuvant effect of Vaxfectin.

NP or β-gal peptide for 5-6 days were assayed for CTL lysis of P815 target cells pulsed with NP or β-gal peptide. Unpulsed P815 cells were used to detect non-specific lysis. The antigen specific CTL effector titration curves for % lysis of peptide pulsed target cells are shown in Figure 12. The results for both NP and β-gal pDNA indicate that formulation of pDNA with Vaxfectin has no significant effect on the CTL response at any of the effector:target ratios tested ($p > 0.05$ at all E:T ratios).

Effect of Vaxfectin on IgG1 and IgG2a antibody titers

The T helper 1 (Th1) type immune responses induced by intramuscular pDNA immunization promote antibody heavy chain switch in responding B cells to the IgG2a sub-isotype (Raz *et al.*, 1996). Thus production of antigen specific IgG2a is greater than antigen specific IgG1. The use of an adjuvant in pDNA vaccines could qualitatively change the immune response, resulting in greater production of either IgG1 or IgG2a. To determine the effect of Vaxfectin on the relative proportion of antigen specific serum IgG1 to IgG2a when formulated with various antigen plasmid DNAs, 6 week sera were analyzed for antigen specific sub-isotype titers.

As shown in Figure 13a, immunizations with naked pDNA encoding different antigens result in sub-isotype profiles that are unique to each antigen. Although the relative proportion of IgG1 and IgG2a varied for different antigens, IgG2a was the predominant sub-isotype produced, consistent with a Th1 type immune response. Vaxfectin formulated with all five model antigen pDNAs results in an increase of both antigen specific antibody sub-isotypes (Table 4). Increases in antigen specific IgG1 and IgG2a were approximately the same magnitude for Vaxfectin formulated pDNA for 4 of the model antigens. As compared to titers obtained with naked pDNA, formulating pDNA with Vaxfectin increased anti-HEL IgG1 titers 9-fold and IgG2a titers 11-fold. Vaxfectin increased anti-β-gal, anti-mouse Id/human Fc and anti-factor IX IgG1 titers 2 to 5-fold and IgG2a titers 2 to 4-fold over naked DNA. Vaxfectin formulated with NP pDNA increased the average anti-NP IgG1 antibody titer by 15-fold over naked pDNA. However, the average anti-NP IgG2a antibody titer was only increased 3-fold. Thus, the relative proportions of IgG1 and IgG2a elicited by

Effect of Vaxfectin on the cytokine profile

The antigen specific antibody sub-isotype analyses suggest that the responses induced with Vaxfectin formulated pDNA, as for naked pDNA are Thl type responses. To confirm that Vaxfectin has no effect on the T helper (Th) cytokine profile in an antigen specific *in vitro* recall response, spleens from groups of mice immunized with NP or β-gal pDNA formulated with or without Vaxfectin were harvested 8-9 weeks following the boost injection. Splenocytes were cultured and stimulated with NP or β-gal protein. Supernatants harvested from the cultured cells were assayed for IFN-γ and interleukin-4 (IL-4) production. Immunizations with NP or β-gal plasmid DNA formulated with or without Vaxfectin resulted in IFN-γ production in splenocyte cultures from all groups of immunized mice (Figure 14). Low levels of IL-4 were produced in all groups of mice; however, IFN-γ was the predominant cytokine produced, suggesting a Thl biased response.

In summary, the foregoing examples demonstrate the robust adjuvant effects of a unique cationic lipid-based formulation for nucleic acid vaccines. The stimulation of the humoral response can be accomplished without diminishing the strong cytolytic responses typical of nucleic acid-based vaccines. The adjuvant activity is seen in both mice and rabbits, thus implying the pharmaceutical applications in other mammals, as well as offering potential benefit in nucleic acid-based preparation of monoclonal and polyclonal antibodies. GAP-DMORIE/co-lipid (e.g., Vaxfectin) mediated enhancement of the antibody responses was readily observed after a single unilateral intramuscular injection. This is important for the immunization of farm animals where single-shot vaccines are highly desirable since roundup of range animals is expensive and can result in loss of production due to stress (Beard, C.W., *el al, Nat. Biotechnol 16(13)*:1325-1328 (1998)).

Example 5

Human Administration

Immunogenic compositions comprising pDNA encoding hemagglutinin(HA), mixed with an adjuvant containing GAP-DMORIE formulated as a 1:1 (mol:mol)

What Is Claimed Is:

1. An adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and one or more co-lipids.

2. The adjuvant composition of claim 1 wherein the co-lipid is a neutral lipid.

3. The adjuvant composition of claim 1 wherein the co-lipid is a phosphatidylethanolamine.

4. The adjuvant composition of claim 3 wherein the phosphatidylethanolamine is selected from the group consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE).

5. The adjuvant composition of claim 4 wherein the phosphatidylethanolamine is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).

6. The adjuvant of claim 4 wherein the phosphatidylethanolamine is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

7. The adjuvant of claim 1 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 9:1 to about 1:9.

8. The adjuvant of claim 1 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 4:1 to about 1:4.

9. The adjuvant of claim 1 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 2:1 to about 1:2.

10. The adjuvant of claim 1 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of about 1:1.

11. The adjuvant of claim 6 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of from about 2:1 to about 1:2.

12. The adjuvant of claim 6 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of about 1:1.

13. An immunogenic composition comprising an immunogen and an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and one or more co-lipids.

14. The immunogenic composition of claim 13 wherein the immumogen comprises an immunogen-encoding polynucleotide.

15. The immunogenic composition of claim 14 wherein the immunogen-encoding polynucleotide is DNA, RNA, or nucleic acid oligomer.

16. The immunogenic composition of claim 14 wherein the immunogen-encoding polynucleotide is a linear or circular polynucleotide.

17. The immunogenic composition of claim 14 wherein the immunogen-encoding polynucleotide is all or part of a plasmid DNA.

18. The immunogenic composition of claim 14 wherein the co-lipid is selected from the group consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE).

19. The immunogenic composition of claim 18 wherein the co-lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).

20. The immunogenic composition of claim 18 wherein the co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

21. The immunogenic composition of claim 14 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 9:1 to about 1:9.

22. The immunogenic composition of claim 14 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 4:1 to about 1:4.

23. The immunogenic composition of claim 14 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium

(GAP-DMORIE) and the co-lipid are in molar ratio of from about 2:1 to about 1:2.

24. The immunogenic composition of claim 14 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of about 1:1.

25. The immunogenic composition of claim 20 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of from about 2:1 to about 1:2.

26. The immunogenic composition of claim 20 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of about 1:1.

27. A method for immunizing a vertebrate comprising administering into a tissue or cavity of said vertebrate an immunogenic composition comprising one or more immunogen-encoding polynucleotides and an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE), wherein an immunogen is expressed in the vertebrate in an amount sufficient to generate an immune response to the inummogen.

28. The method of claim 27 wherein the immunogenic composition further comprises one or more co-lipids.

29. The method of claim 28 wherein the immunogen-encoding polynucleotide is DNA, RNA, or nucleic acid oligomer.

30. The method of claim 28 wherein the immunogen-encoding polynucleotide is all or part of a plasmid DNA.

31. The method of claim 28 wherein the co-lipid is selected from the group consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE).

32. The method of claim 28 wherein the co-lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).

33. The method of claim 28 wherein the co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-

phosphoethanolamine (DPyPE).

34. The method of claim 28 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 9:1 to about 1:9.

35. The method of claim 28 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 4:1 to about 1:4.

36. The method of claim 28 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of from about 2:1 to about 1:2.

37. The method of claim 28 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and the co-lipid are in molar ratio of about 1:1.

38. The method of claim 33 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of from about 2:1 to about 1:2.

39. The method of claim 33 wherein the salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of about 1:1.

40. The method of claim 27 wherein the vertebrate is a mammal.

41. The method of claim 40 wherein the mammal is a human.

42. The method of claim 28 wherein the immunogenic composition is a pharmaceutical composition.

43. The method of claim 28 wherein said immunogen is selected from the group consisting of a bacterial polypeptide, a fungal polypeptide, a parasite polypeptide, an allergenic polypeptide, a tumor specific polypeptide, and immunogenic fragments, derivatives, or analogs thereof.

44. The method of claim 28 wherein said tissue is selected from the group consisting of muscle, skin, brain tissue, lung tissue, liver tissue, spleen tissue, bone

marrow tissue, thymus tissue, heart tissue, lymph tissue, blood tissue, bone tissue, connective tissue, mucosal tissue, pancreas tissue, kidney tissue, gall bladder tissue, stomach tissue, intestinal tissue, testicular tissue, ovarian tissue, uterine tissue, vaginal tissue, rectal tissue, nervous system tissue, eye tissue, glandular tissue, and tongue.

45. The method of claim 28 wherein said cavity is selected from the group consisting of lung, mouth, nasal cavity, stomach, peritoneum, intestine, heart chamber, vein, artery, capillary, lymphatic, uterus, vagina, rectum, and ocular cavity.

46. The method of claim 28, wherein said cavity comprises a mucosal surface.

47. The method of claim 28, wherein said tissue is muscle.

48. The method of claim 47, wherein said tissue is skeletal muscle.

49. The method of claim 28, whercin said administration is intravenous.

50. The method of claim 28, wherein said administration is by a route selected from the group consisting of intramuscular, intratracheal, intranasal, transdermal, interdermal, subcutaneous, intraocular, vaginal, rectal, intraperitoneal, intraintestinal and inhalation.

51. The method of any one of claim 28, wherein said administration is mediated by a device selected from the group consisting of a particle accelerator, a pump, an intradermal applicator, a biolistic injector, a pneumatic injector, a sponge depot, a pill and a tablet.

52. The method of claim 28, wherein said administration is mediated by a CO_2 powered jet injection system.

53. A method for providing to a mammal a prophylactic or therapeutic treatment associated with a bacterial infection comprising

administering to the mammal an immunogenic composition comprising one or more immunogen-encoding polynucleotides associated with the bacterial infection and an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and a co-lipid, wherein an immunogen is expressed in the mammal in an amount sufficient to generate an immune response to the immunogen.

54. The method of claim 53 wherein the co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

55. A method for providing to a mammal a prophylactic or therapeutic treatment associated with a viral infection comprising

administering to the mammal an immunogenic composition comprising one or more immunogen-encoding polynucleotides associated with the viral infection and an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and a co-lipid, wherein an immurogen is expressed in the mammal in an amount sufficient to generate an immune response to the immunogen.

56. The method of claim 55 wherein the co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

57. A method for providing to a mammal a prophylactic or therapeutic treatment associated with an abnormal growth of a cell population comprising

administering to the mammal an immunogenic composition comprising one or to more immunogen-encoding polynucleotides associated with the abnormal growth of the cell population and an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and a co-lipid, wherein an immunogen is expressed in the mammal in an amount sufficient to generate an immune response to the immunogen.

58. The method of claim 57 wherein the abnormal growth of a cell population is associated with cancer.

59. The method of claim 58 wherein the co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

60. A pharmaceutical kit comprising:

(a) a container holding 1 ng to 30 mg of an immunogen-encoding polynucleotide which operably encodes an immunogen within vertebrate cells in vivo; and

(b) an adjuvant composition comprising a salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and a co-lipid,

whereby said immunogen is provided in a prophylactically or therapeutically effective amount to treat a vertebrate.

61. The pharmaceutical kit of claim 60, wherein (b) is in the container of (a).

62. The pharmaceutical kit of claim 60, wherein (b) is in a separate container from (a).

63. The pharmaceutical kit of claim 60, further comprising an administration means.

64. The pharmaceutical kit of claim 60, wherein said co-lipid is 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE).

65. The pharmaceutical kit of claim 64, wherein said salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of about 2:1 to about 1:2.

66. The pharmaceutical kit of claim 64, wherein said salt of (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium (GAP-DMORIE) and 1,2-diphytamoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) are in molar ratio of about 1:1.

WO 00/57917
PCT/US00/08282

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:
NAN, Wu
Pillsbury Madison & Sutro LLP
50 Fremont Street
San Francisco, CA 94105
ETATS-UNIS D'AMERIQUE

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 05 October 2000 (05.10.00)	
Applicant's or agent's file reference ~~90243/126323~~ 1530.031PC02	**IMPORTANT NOTICE**
International application No. PCT/US00/08282	**International filing date** (day/month/year) 24 March 2000 (24.03.00)
Priority date (day/month/year) 26 March 1999 (26.03.99)	
Applicant VICAL INCORPORATED et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA,EP,JP

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   05 October 2000 (05.10.00) under No. WO 00/57917

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

Chpt II demand 10/26/00
Chpt I N.P. (11/26/00) 10-26-00

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38